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14. ABSTRACT

Progress has been made on identifying biosensors that will be used to report on the fermentation yields of industrially relevant biological compounds. Screening of the desired chemicals, and sequencing and annotation of isolated microbes was completed previously. Construction of reporter plasmids for testing candidate transcription factors from sequenced microbes and that respond to the desired chemicals was completed previously. During this period we have optimized the genetic architecture of the reporter system to increase its dynamic range (Figure 1). Similarly, we have optimized the screening process so that empirical verification of transcription factor candidates is far less labor intensive. The genomes of 13 microbes (that utilize desired chemicals as the sole carbon and energy source) have been extensively evaluated for the presence of probable chemical-transcription factor pairs. For eight of these there is a high-quality candidate. We expect roughly the same ratio once the remaining genomes are analyzed (over the next month) and should easily surpass the 5-10 transcription factor candidates we expected to be able to process for this proposal. Moreover, the labor surrounding transcription factor characterization has been dramatically reduced. It therefore seems likely that we will be able to evaluate all candidates (except for those that prove to be difficult to assemble with the reporter system).

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J. Craig Venter™
INSTITUTE

Rapid parallel screening for strain optimization

Report Title:	Quarterly R&D Status Report
Report Number:	HR0011-12-C-0062.6
Reporting Period:	17AUG13 to 16NOV13
Contract No.:	HR0011-12-C-0062
Performing Organization:	J. Craig Venter Institute 9704 medical Center Drive Rockville, MD 20850 USA
Principal Investigator:	Chuck Merryman

Abstract

Progress has been made on identifying biosensors that will be used to report on the fermentation yields of industrially relevant biological compounds. Screening of the desired chemicals, and sequencing and annotation of isolated microbes was completed previously. Construction of reporter plasmids for testing candidate transcription factors from sequenced microbes and that respond to the desired chemicals was completed previously. During this period we have optimized the genetic architecture of the reporter system to increase its dynamic range (Figure 1). Similarly, we have optimized the screening process so that empirical verification of transcription factor candidates is far less labor intensive. The genomes of 13 microbes (that utilize desired chemicals as the sole carbon and energy source) have been extensively evaluated for the presence of probable chemical-transcription factor pairs. For eight of these there is a high-quality candidate. We expect roughly the same ratio once the remaining genomes are analyzed (over the next month) and should easily surpass the 5-10 transcription factor candidates we expected to be able to process for this proposal. Moreover, the labor surrounding transcription factor characterization has been dramatically reduced. It therefore seems likely that we will be able to evaluate all candidates (except for those that prove to be difficult to assemble with the reporter system).

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Summary

In total, 108 compounds have been used for enrichment culture and 85 compounds produced colonies (when used as the sole source of carbon and energy). These have been sequenced and the genomes sequenced and annotated. Close scrutiny of 13 genomes has revealed the presence of eight transcription-factor candidates that likely respond to the respective chemicals. Constructs for cloning and evaluating transcription factors were completed previously. This reporter system has now been optimized to increase the dynamic range and improve throughput. Trial runs have been completed with a cumate-responsive reporter system and are being analyzed.

Introduction

The overall goal in this contract is to link cell-based production to cell survival and thereby make the engineering of new microbial strains that produce industrially relevant biochemicals routine. Recent synthetic biology techniques can make billions of variant cells. Although, many potentially informative mutants are easily made, product yield can only be determined in a few of these. The majority of industrially relevant biomolecules are not chromophores, naturally discernible, or otherwise easily detected. Nevertheless, genetic circuits are capable of linking chemical production to discernible signals such as growth or color intensity. Such a system would allow numerous mutants and mutant combinations to be examined quickly. Genetic circuits needed to screen mutant populations in parallel rely upon the availability of an appropriate biosensor that activates a reporter gene in a product dependent fashion. In this project, genes for two-component and one-component signaling systems (that respond to industrially relevant biomolecules) are identified. To demonstrate that such sensors can be used to maximize product yield, one sensing system will be further engineered. We will reformat this sensor so that it drives expression of a reporter such as an antibiotic resistance marker. This sensor/resistance cassette, and a biosynthetic pathway capable of producing the molecule to which the sensor responds, will be placed within a heterologous host that does not have an overlapping pathway. Basal synthesis of the targeted chemical (by the orthogonal biosynthetic pathway) activates the sensor and increases transcription of the resistance marker (i.e. reporter). In other words, the fermentation product is also the sensor ligand and thus, biosynthesis drives production of the reporter and cell phenotype. Targeted, genome-wide and barcoded alterations to the host genome will then be installed. Variants with better and better chemical production are selected by virtue of increased reporter activity.

Methods, Assumptions and Procedures

During this quarter we were slated to experimentally validate 5-10 candidate transcription factors by measuring their response to exogenously supplied substrates (i.e. task 5). Some of the work necessary for this task was delayed due to backordered material from a manufacturer. We are nearly now back on track and expect to complete this task in the upcoming quarter. The modular assembly vector we have developed in the meantime places an ssrA-tagged GFP reporter under control of the T5 promoter and the transcription factor under constitutive expression. The ssrA-tag promotes degradation of the reporter. Qualitatively this appears to increase the signal to noise ratio using a cumate-responsive transcription factor and complete analysis is underway. Moreover, automated recording of GFP fluorescence in 96-well plates is less labor intensive than evaluating cell growth rates under antibiotic selection. Thus, we now expect to be able to examine all high-quality transcription factor candidates rather than 5-10. We also expect that the output of these experiments will be more definitive due to the increase in signal to noise.

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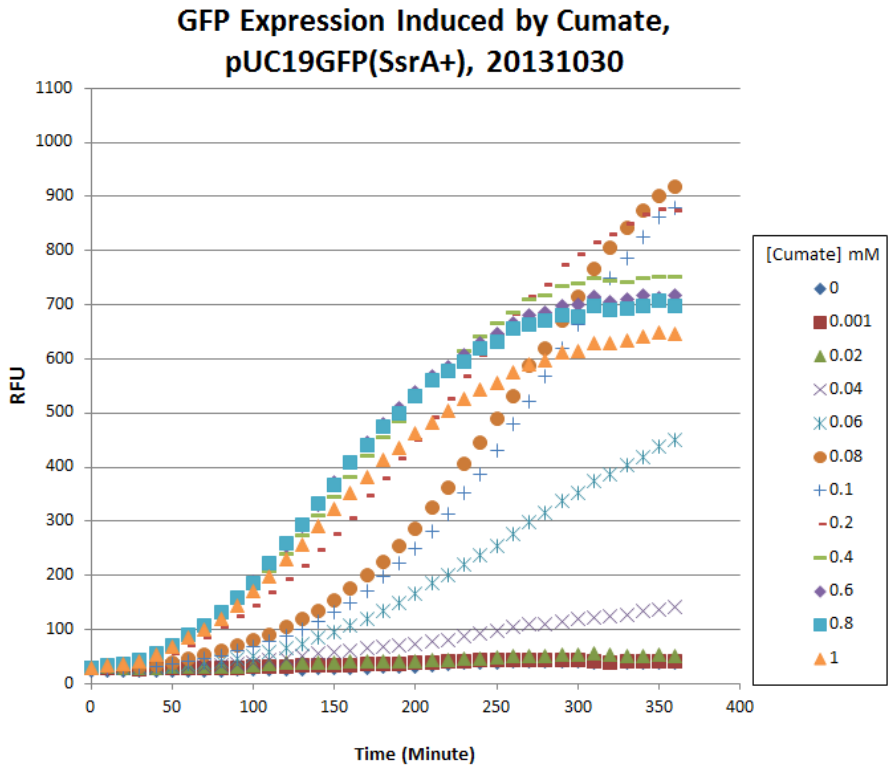
Results and Discussion

Screening of chemicals, processing of the resulting microbes, and construction of necessary plasmids etc., was completed previously. During this period we have improved the dynamic range of the previous reporter system another 3-fold with the final signal being about 30-fold over background. We have also decreased the labor necessary to characterize transcription-factor candidates by employing an automated system for recording results. The genomes of 13 microbes have been fully evaluated for appropriate transcription factors and we identified 8 high-quality candidates. As the rest of the genomes are processed over the next month, we expect to collect about 40-50 such candidates in all. Because the new GFP-based reporter system requires less labor, over the next quarter we will likely be able to examine all candidates rather than the predicted 5-10.



Conclusions

The results indicate that a chemical made by one organism is likely to be used as food by some other microbe. Bacteria typically utilize the most efficient carbon source available (glucose often being the preferred substrate). More exotic carbon sources are generally subject to catabolite repression and systems for their utilization are activated after preferred carbon sources are exhausted. Besides catabolite repression, sensors are often employed so that the appropriate degradation pathway for a non-preferred carbon source is activated. Our sequencing results have identified organisms rich in transcription-factor based sensors that are integrated with appropriate catabolic gene clusters. The next step is to experimentally test individual sensors for their ability to respond to a specified carbon source. This will set the stage for downstream work on the overproduction of such molecules. Identification and experimental validation of specific sensors is indispensable but current results are promising: 1) most target chemicals (~80%) readily produce microbial growth, 2) colony morphology, etc., suggests that different target chemicals resulted in the isolation of different microbial species, 3) automatable procedures can be used if high-throughput screens are needed in the future, 4) about 70% of recovered organisms are from the genus *Pseudomonas*, consistent with the commonplace identification of sensors and degradation pathways within this genus when anthropogenic chemicals are used as targets, 5) fully sequenced and annotated genomes are rich in transcription factors, 6) the first 13 exhaustively analyzed genomes produced 8 transcription-factors that are clearly analogous to known factors that respond to chemicals related to those used experimentally, and, 7) a high-throughput genetic system for evaluating chemical-transcription factor pairs behaves well over about a ~30-fold dynamic range.

Statement of Work Task List:

- Task 1 (Phase I, Year 1, Months 0-3): Completed (please refer to report HR0011-12-C-2.1)
- Task 2 (Phase I, Year 1, Months 4-9): Completed. Sixty-five isolates have been sequenced.
- Task 3 (Phase I, Year 1, Months 10-12): Completed. Selected microbes have been sequenced, and annotated.
- Task 4, (Phase I, Year 1, months 12). Nearly completed. Ranking of transcription factors for evaluation was delayed by delays in sequencing genomes. A full ranked list will be available over the next month.
- Task 5 (Phase II, Year 2, Months 13-18): Initiated and optimized. Construction and testing of the reporter system has been completed and an automated process was produced during sequencing delays. We now expect to be able to process more than the 5-10 original candidates.

Planned Activities for the Next Reporting Period

During the next reporting period we will finalize all bioinformatics, process candidate transcription factors.

Program Financial Status

In Process & Completed Tasks	Planned Expend	Actual Expend (Cumulative to Date)	% Budget Completion	At Completion	Latest Revised Estimate	Remarks
Task 1	$59,251	$59,251	100%	$59,251	$59,251	Completed
Task 2	$69,229	$69,229	100%	$69,229	$69,229	Completed
Task 3	$124,706	$124,706	100%	N/A	$124,706	Completed
Task 4	$255,817	$235,814	92%	N/A	$255,817	Nearly Complete
Task 5	$255,817	$42,666	17%	N/A	$255,817	In Progress
Cumulative	$764,820	$531,666	69%	N/A	$764,820	N/A

There is no management reserve or unallocated resources.

Based on the currently authorized work:

- Is current funding sufficient for the current fiscal year? Yes
- What is the next fiscal year funding requirement at current anticipated levels? The budgeted amount for Year 2 of the project is $396,905.25.
- Have you included in the report narrative any explanation of the above data and are they cross-referenced? Not applicable; current funding is sufficient for the current fiscal year.